

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

VOSSIUS & PARTNER
Siebertstr. 4
D-81675 München
ALLEMAGNE

Date of mailing (day/month/year) 05 April 2001 (05.04.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference C 1748 PCT	
International application No. PCT/EP99/07633	International filing date (day/month/year) 12 October 1999 (12.10.99)

1. The following indications appeared on record concerning:	
<input checked="" type="checkbox"/> the applicant <input type="checkbox"/> the inventor <input type="checkbox"/> the agent <input type="checkbox"/> the common representative	
Name and Address	State of Nationality
	State of Residence
	Telephone No.
	Facsimile No.
Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:	
<input checked="" type="checkbox"/> the person <input checked="" type="checkbox"/> the name <input checked="" type="checkbox"/> the address <input checked="" type="checkbox"/> the nationality <input checked="" type="checkbox"/> the residence	
Name and Address BERGER, ERIKA Ursulastrasse 22 50767 Köln Germany	State of Nationality DE
	State of Residence DE
	Telephone No.
	Facsimile No.
Teleprinter No.	
3. Further observations, if necessary: Heiress of deceased inventor BERGER, Dieter.	
4. A copy of this notification has been sent to:	
<input checked="" type="checkbox"/> the receiving Office <input type="checkbox"/> the designated Offices concerned <input type="checkbox"/> the International Searching Authority <input checked="" type="checkbox"/> the elected Offices concerned <input type="checkbox"/> the International Preliminary Examining Authority <input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Céline Faust Telephone No.: (41-22) 338.83.38
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	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input checked="" type="checkbox"/> the person	<input checked="" type="checkbox"/> the name	<input checked="" type="checkbox"/> the address <input checked="" type="checkbox"/> the nationality <input checked="" type="checkbox"/> the residence
Name and Address BERGER, Werner Am Rosengarten 15 50827 Köln Germany	State of Nationality DE	State of Residence DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary: Heir of deceased inventor BERGER, Dieter.		
4. A copy of this notification has been sent to:		
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<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Céline Faust
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input checked="" type="checkbox"/> the person	<input checked="" type="checkbox"/> the name	<input checked="" type="checkbox"/> the address <input checked="" type="checkbox"/> the nationality <input checked="" type="checkbox"/> the residence
Name and Address LISSO, Janina Mittelweg 28 06862 Roßlau Germany	State of Nationality DE	State of Residence DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary: Heiress of deceased inventor BERGER, Dieter.		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Céline Faust
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 May 2000 (03.05.00)	
International application No. PCT/EP99/07633	Applicant's or agent's file reference C 1748 PCT
International filing date (day/month/year) 12 October 1999 (12.10.99)	Priority date (day/month/year) 12 October 1998 (12.10.98)
Applicant BERGER, Dieter et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

04 April 2000 (04.04.00)

☐ in a notice effecting later election filed with the International Bureau on:
2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

F. Baechler

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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Administrative Instructions, Section 422)

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Siebertstr. 4
D-81675 München
ALLEMAGNE

Date of mailing (day/month/year) 12 April 2000 (12.04.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference C 1748 PCT	
International application No. PCT/EP99/07633	International filing date (day/month/year) 12 October 1999 (12.10.99)

1. The following indications appeared on record concerning:		
<input checked="" type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input type="checkbox"/> the agent <input type="checkbox"/> the common representative
Name and Address PLANTTEC BIOTECHNOLOGIE GMBH Forschung & Entwicklung Hermannswerderstr. 14 D-14473 Potsdam Germany	State of Nationality DE	State of Residence DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
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Name and Address PLANTTEC BIOTECHNOLOGIE GMBH Forschung & Entwicklung Hermannswerder 14 D-14473 Potsdam Germany	State of Nationality DE	State of Residence DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input checked="" type="checkbox"/> the designated Offices concerned	
<input checked="" type="checkbox"/> the International Searching Authority	<input type="checkbox"/> the elected Offices concerned	
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Céline Faust
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

09/806767

JCO8 Rec'd PCT/PTO 03 APR 2001
PATENT
0147-0223P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: BERGER, Dieter et al.
Int'l. Appl. No.: PCT/EP99/07633
Appl. No.: New Group:
Filed: April 3, 2001 Examiner:
For: MEANS AND METHODS FOR MODULATING
STOMATA CHARACTERISTICA IN PLANTS

LETTER

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

April 3, 2001

Sir:

The PTO is requested to use the amended sheets/claims attached hereto
(which correspond to Article 34 amendments or to claims attached to the
International Preliminary Examination Report) during prosecution of the above-
identified national phase PCT application.

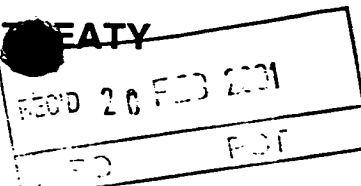
Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By *Leonard R. Svensson* #36623
Leonard R. Svensson, #30,330

LRS/cqc
0147-0223P

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference C 1748 PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP99/07633	International filing date (day/month/year) 12/10/1999	Priority date (day/month/year) 12/10/1998
International Patent Classification (IPC) or national classification and IPC C12N15/57		
Applicant MAX-PLANCK-GESELLSCHAFT ...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 8 sheets, including this cover sheet.

- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 04/04/2000	Date of completion of this report 16.02.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer BULCAO DE MELO ..., T Telephone No. +49 89 2399 8972 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/07633

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:

Description, pages:

1-44 as originally filed

Claims, No.:

1-21 as received on 29/01/2001 with letter of 29/01/2001

Drawings, sheets:

1/9-9/9 as originally filed

Sequence listing part of the description, pages:

1-43, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
 - ☐ the language of publication of the international application (under Rule 48.3(b)).
 - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:
- ☒ contained in the international application in written form.
 - ☒ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority in written form.
 - ☐ furnished subsequently to this Authority in computer readable form.
 - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
4. The amendments have resulted in the cancellation of:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/07633

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 1-21 (partially).

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☒ no international search report has been established for the said claims Nos. 1-21 (partially).

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

- ☐ the written form has not been furnished or does not comply with the standard.
- ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/07633

1. Statement

Novelty (N)	Yes:	Claims	4 and 7-21
	No:	Claims	1-3, 5 and 6
Inventive step (IS)	Yes:	Claims	7-10, 14 and 19-21
	No:	Claims	4, 11-13 and 15-18
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

SECTION I

1. The amended **claims 1-21** filed with your letter of 29.01.01 are considered to be allowable under **Rule 70.2 (c) PCT**.

SECTION III

2. The present International Preliminary Examination Report is restricted to the searched subject-matter, i.e.
Claims 1-21 (partially), which refer to a DNA molecule having sequence SEQ ID NO:1, the encoded subtilisin-like serine protease having sequence SEQ ID NO:2, their use and transgenic plants with altered activity of the above protease.

SECTION V

3. Reference is made to the following documents:

D1: EMBL Database, Accession Number AC002411, 1997

D2: STRPEMBL Database, Accession Number 064495, August 1998

D3: Journal of Experimental Botany, Vol. 49, abstract P7.58, 1998, page 62

4. Novelty (**Article 33(2) PCT**)

The present application does not satisfy the criterion set forth in **Article 33 (2) PCT** because the subject-matter of **claims 1-3, 5 and 6** is considered to be part of the prior art as defined in the regulations (**Rule 64 (1)-(3) PCT**).

Document **D1** discloses the sequence of the Arabidopsis thaliana F20D22 protein and of its encoding nucleic acid. It should be noted that the F20D22 protein corresponds to the SDD1 protein, which has sequence SEQ ID NO:2.

Document **D2** also discloses the sequence of the Arabidopsis thaliana F20D22 protein.

Therefore, the subject-matter of **claims 1-3** is not novel over **D1 and D2**.

Although not explicitly disclosed, the subject-matter of **claims 5 and 6** is also anticipated by documents **D1 and D2**.

Regarding the above lack of novelty the following should be considered:

The feature "recombinant" (**claims 1-3**) is not regarded as a distinguishing feature and therefore it does not constitute a genuine restriction on the DNA molecule. On the contrary, a recombinant DNA molecule (encoding a protein having SEQ ID NO:2) includes both the native DNA molecule and the recombinantly produced DNA molecule. Said term only refers to the process by which the DNA molecule is obtained.

A DNA molecule, regarded as a chemical product, should be clearly and unambiguously characterised by technical features and not by its process of manufacture.

Regarding this issue, it should be pointed out that "claims for products defined in terms of a process of manufacture are admissible only if the products as such fulfil the requirements for patentability...", which is not the case here (see above). Moreover "a product is not rendered novel merely by the fact that it is produced by means of a new process".

Therefore, the fact that the DNA molecules of the present application are obtained by recombinant DNA technology does not render them novel.

5. Inventive Step (Article 33 (3) PCT)

5.1. Claims 7-10, 14 and 19-21

The **technical problem** to be solved by **claims 7-10, 14 and 19-21** can be considered to lie in the provision of means and methods for modulating the stomatal density in plants.

The **solution** provided by the Applicant to solve the above problem relies in the use of a DNA molecule (SEQ ID NO:1) encoding a subtilisin-like serine protease (SEQ ID NO:2).

The **closest prior art** to evaluate the inventiveness of **claims 7-10, 14 and 19-21** is document **D2**, which discloses the sequence of the Arabidopsis thaliana F20D22 protein (=SDD1 protein). D2 further refers to a subtilisin function of said protein.

Although document **D3** discloses the *Arabidopsis thaliana* ARA12 protein, a subtilisin-like serine protease, and the use of its encoding cDNA in the antisense orientation to transform *Arabidopsis* plants, it further states that the function of said protease remains unknown.

D3 is not regarded as an incentive for the use of the sequence of D2 (or D1) for the production of transgenic plants with altered stomata characteristics, since it was not expected that the introduction of said DNA into a plant would lead to said phenotype. Therefore, the solution for the above problem was not obvious for the person skilled in the art.

Thus, the subject-matter of **claims 7-10, 14 and 19-21** is considered to involve an inventive step.

5.2. Claims 11-13, 15 and 16

The present application does not satisfy the criterion set forth in **Article 33 (3) PCT** because the subject-matter of **claims 11-13, 15 and 16** does not involve an inventive step (**Rule 65 (1) and (2) PCT**).

The **closest prior art** to evaluate the inventiveness of **claims 11-13, 15 and 16** is document **D2**, which discloses the sequence of the *Arabidopsis thaliana* F20D22 protein (=SDD1 protein). D2 further refers to a subtilisin function of said protein.

Document **D3** discloses the *Arabidopsis thaliana* ARA12 protein, a subtilisin-like serine protease, and the use of its encoding cDNA in the antisense orientation to provide a construct to transform *Arabidopsis* plants.

In view of the above, the person skilled in the art would not require any inventive skills in order to perform the experiments disclosed in D3 with another subtilisin-like serine protease, namely the one disclosed in D2 (or D1), and thereby provide subtilisin deficient transgenic plants.

Therefore, the subject-matter of **claims 11-13, 15 and 16** does not involve an inventive step.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP99/07633

5.3. Claims 4, 17 and 18

Claims 4, 17 and 18 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step.

6. Industrial Applicability (Article 33(4) PCT)

The subject-matter of present **claims 1-21** is susceptible of industrial applicability as defined in **Article 33 (4) PCT**.

SECTION VII

7. Contrary to the requirements of **Rule 5.1(a)(ii) PCT**, the relevant background art disclosed in documents **D1-D4** are not mentioned in the description, nor are these documents identified therein.

PCT/EP99/07633
MAX-PLANCK-GESELLSCHAFT...et al.
Our Ref.: C 1748 PCT

Claims

1. A recombinant DNA molecule comprising:
 - (i) a nucleic acid molecule encoding a subtilisin-like serine protease or encoding a biologically active fragment of such a protein, selected from the group consisting of
 - (a) nucleic acid molecules comprising a nucleotide sequence encoding a protein comprising the amino acid sequence as given in SEQ ID NO: 2, 8, 10 or 12;
 - (b) nucleic acid molecules comprising a nucleotide sequence as given in SEQ ID NO: 1, 7, 9 or 11;
 - (c) nucleic acid molecules encoding a protein comprising at least the D region, H region, substrate binding site and/or S region of the subtilisin-like serine protease encoded by a nucleic acid molecule of (a) or (b); or
 - (d) nucleic acid molecules hybridizing with the complementary strand of a nucleic acid molecule as defined in any one of (a) to (c);
 - (e) nucleic acid molecules encoding a protein the amino acid sequence of which is at least 65% identical to the amino acid sequence encoded by a nucleic acid molecule of any one of (a) to (c);
 - (f) nucleic acid molecules, the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (e); or
 - (ii) a nucleic acid molecule encoding a mutant non-active or a hyper-active form of or an antibody against the subtilisin-like serine protease encoded by a nucleic acid molecule of (i); or

- (iii) a nucleic acid molecule which specifically hybridizes with a nucleic acid molecule of (i) or the complementary strand thereof.
2. The recombinant DNA molecule of claim 1 wherein the nucleic acid molecule is DNA, cDNA, genomic DNA or synthetically synthesized DNA.
 3. The recombinant DNA molecule of claim 1 wherein the nucleic acid molecule is derived from a plant, preferably Arabidopsis or potato.
 4. The recombinant DNA molecule of any one of claims 1 to 3 wherein said nucleic acid molecule is operably linked to regulatory elements allowing the expression of the nucleic acid molecule in plants.
 5. A vector comprising a recombinant DNA molecule of any one of claims 1 to 4.
 6. A host cell containing a vector of claim 5 or a recombinant DNA molecule of any one of claims 1 to 4.
 7. A method for the production of transgenic plants with altered stomata characteristics compared to wild type plants comprising the introduction of a recombinant DNA molecule of any one of claims 1 to 4 or the vector of claim 5 into the genome of a plant, plant cell or plant tissue.
 8. A transgenic plant cell comprising stably integrated into the genome a recombinant DNA molecule of any one of claims 1 to 4 or a vector of claim 5 or obtainable according to the method of claim 7, wherein the expression of the nucleic acid molecule results in an increased expression or activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.
 9. A transgenic plant or a plant tissue comprising plant cells of claim 8.

10. The transgenic plant of claim 9 which displays a decreased stomata density, lower conductance of stomata and/or wherein the water consumption is lowered compared to wild type plants.
11. A transgenic plant cell which contains stably integrated into the genome a recombinant DNA molecule of any one of claims 1 to 4 or part thereof, a vector of claim 5 or obtainable according to the method of claim 7, wherein the presence, transcription and/or expression of the nucleic acid molecule or part thereof leads to reduction of the synthesis or the activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.
12. The plant cell of claim 11, wherein the reduction is achieved by an antisense, sense, ribozyme, co-suppression and/or dominant mutant effect.
13. A transgenic plant or plant tissue comprising the plant cells of claim 11 or 12.
14. The transgenic plant of claim 13 which displays increased stomatal density and/or higher conductance of stomata and/or increased content of sugars and/or protein in plant leaves compared to wild type plants.
15. The transgenic plant of any one of claims 9, 10, 13 or 14, the plant cell of any one of claims 8, 11 or 12, or the plant tissue of claim 9 or 13, wherein said plant, plant cell or plant tissue is derived from a monocotyledonous or dicotyledonous plant.
16. The transgenic plant, plant cell or plant tissue of claim 15, wherein said plant is derived from maize, rice, barley, wheat, rye, oats, tomato, melon, banana, chicoree, salad, cabbage, potato, tobacco, alfalfa, clover, oilseed rape, sunflower, peanut, soybean, cotton, sugar beet, linseed, flax, millet, hemp, sugar cane, bean, pea or tree.
17. Harvestable parts or propagation material of plants of any one of claims 9, 10, 13 or 14 to 16 comprising plant cells of claim 8, 11, 12, 15 or 16.

18. A kit comprising a recombinant DNA molecule of any one of claims 1 to 4 or a vector of claim 5.
19. A method for the production of a transgenic plant comprising an increased yield and/or increased stomatal density compared to wild type plants, wherein
 - (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in a decreased activity of a subtilase;
 - (b) a plant is regenerated from the cell prepared according to step (a); and
 - (c) further plants, if any, are generated from the plant prepared according to step (b).
20. A method for the production of a transgenic plant having a decreased water consumption and/or decreased stomatal density compared to wild type plants wherein
 - (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in an increased activity of a subtilase;
 - (b) a plant is regenerated from the cell prepared according to step (a); and
 - (c) further plants, if any, are generated from the plant prepared according to step (b).
21. Use of a nucleic acid molecule encoding or regulating the expression of a subtilisin-like serine protease or a nucleic acid molecule hybridizing with such a nucleic acid molecule, a nucleic acid molecule as defined in any one of claims 1 to 4, a recombinant DNA molecule of any one of claims 1 to 4, or a vector of claim 5 for the production of plants with improved fresh and dry weight, for enhancing the content of sugars and/or protein in plant leaves for the production of plants with reduced leaf temperatures or with reduced water loss and lower water consumption, for the modulation (enhancement) of CO₂ uptake into and H₂O release from leaves, for sustained photosynthesis under high intensity conditions or for the improvement of disease resistance of plants.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

09/806767

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum)

C 1748 PCT

Box No. I TITLE OF INVENTION

Means and methods for modulating stomata characteristic in plants

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

Max-Planck-Gesellschaft zur Förderung
der Wissenschaften e.V.
Berlin
DE

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (that is, country) of nationality:

DE

State (that is, country) of residence:

DE

This person is applicant
for the purposes of:

☐ all designated
States

☒ all designated States except
the United States of America

☐ the United States
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the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

PlantTec Biotechnologie GmbH
Forschung & Entwicklung
Hermannswerderstr. 14
14473 Potsdam
DE

This person is:

☒ applicant only

☐ applicant and inventor

☐ inventor only (If this check-box
is marked, do not fill in below.)

State (that is, country) of nationality:

DE

State (that is, country) of residence:

DE

This person is applicant
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☐ the United States
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the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf
of the applicant(s) before the competent International Authorities as:

☒ agent

☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

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DE

Telephone No.

+49-89-413040

Facsimile No.

+49-89-41304111

Teleprinter No.

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
<i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p> <p>BERGER, Dieter Detmolderstr. 63 10715 Berlin DE</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (that is, country) of nationality: DE	State (that is, country) of residence: DE
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p> <p>ALTMANN, Thomas Roßkastanienstr. 61 14469 Potsdam DE</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input checked="" type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (that is, country) of nationality: DE	State (that is, country) of residence: DE
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (that is, country) of nationality:	State (that is, country) of residence:
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p>Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)</p>	<p>This person is:</p> <p><input type="checkbox"/> applicant only</p> <p><input type="checkbox"/> applicant and inventor</p> <p><input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)</p>
State (that is, country) of nationality:	State (that is, country) of residence:
<p>This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box</p>	
<p><input type="checkbox"/> Further applicants and/or (further) inventors are indicated on another continuation sheet.</p>	

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

Regional Patent

- ☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)


National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | |
|--|--|
| <input checked="" type="checkbox"/> AE United Arab Emirates | <input checked="" type="checkbox"/> LR Liberia |
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> LS Lesotho |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> LT Lithuania |
| <input checked="" type="checkbox"/> AT Austria | <input checked="" type="checkbox"/> LU Luxembourg |
| <input checked="" type="checkbox"/> AU Australia | <input checked="" type="checkbox"/> LV Latvia |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> MD Republic of Moldova |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina | <input checked="" type="checkbox"/> MG Madagascar |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> TZ United Republic of Tanzania |
| <input checked="" type="checkbox"/> BR Brazil | <input checked="" type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> MW Malawi |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> MX Mexico |
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| <input checked="" type="checkbox"/> DE Germany | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> DK Denmark | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> EE Estonia | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> FI Finland | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> GB United Kingdom | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> GD Grenada | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> GE Georgia | <input checked="" type="checkbox"/> SL Sierra Leone |
| <input checked="" type="checkbox"/> GH Ghana | <input checked="" type="checkbox"/> TJ Tajikistan |
| <input checked="" type="checkbox"/> GM Gambia | <input checked="" type="checkbox"/> TM Turkmenistan |
| <input checked="" type="checkbox"/> HR Croatia | <input checked="" type="checkbox"/> TR Turkey |
| <input checked="" type="checkbox"/> HU Hungary | <input checked="" type="checkbox"/> TT Trinidad and Tobago |
| <input checked="" type="checkbox"/> ID Indonesia | <input checked="" type="checkbox"/> UA Ukraine |
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| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> ZA South Africa |
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Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

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Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM					<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:			
		national application: country	regional application: regional Office	international application: receiving Office	
item (1) (12.10.1998) 12 October 1998	98 11 9244.6		EP		
item (2)					
item (3)					
<input checked="" type="checkbox"/> The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): <u>(1)</u>					
<i>* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.</i>					
Box No. VII INTERNATIONAL SEARCHING AUTHORITY					
Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):		Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):			
ISA / EP		Date (day/month/year) Number Country (or regional Office)			
Box No. VIII CHECK LIST; LANGUAGE OF FILING					
This international application contains the following number of sheets:		This international application is accompanied by the item(s) marked below:			
request : 4		1. <input type="checkbox"/> fee calculation sheet			
description (excluding sequence listing part) : 44		2. <input type="checkbox"/> separate signed power of attorney			
claims : 4		3. <input type="checkbox"/> copy of general power of attorney; reference number, if any:			
abstract : 1		4. <input type="checkbox"/> statement explaining lack of signature			
drawings : 9		5. <input type="checkbox"/> priority document(s) identified in Box No. VI as item(s):			
sequence listing part of description : 43		6. <input type="checkbox"/> translation of international application into (language):			
Total number of sheets : 105		7. <input type="checkbox"/> separate indications concerning deposited microorganism or other biological material			
		8. <input checked="" type="checkbox"/> nucleotide and/or amino acid sequence listing in computer readable form			
		9. <input type="checkbox"/> other (specify):			
Figure of the drawings which should accompany the abstract:		Language of filing of the international application: <u>English</u>			
Box No. IX SIGNATURE OF APPLICANT OR AGENT					
Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).					
Munich, October 12, 1999  Dr. Hans-Rainer Jaenichen European Patent Attorney					

For receiving Office use only	
1. Date of actual receipt of the purported international application:	2. Drawings: <input type="checkbox"/> received: <input type="checkbox"/> not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): <u>ISA /</u>	6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.

For International Bureau use only	
Date of receipt of the record copy by the International Bureau:	

This sheet is not of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's
file reference

C 1748 PCT

Applicant Max-Planck-Gesellschaft zur Förderung
der Wissenschaften e.V. et al.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE EUR 102.00 T

2. SEARCH FEE EUR 945.00 S

International search to be carried out by EP
(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 105 sheets.

first 30 sheets EUR 413.00 b1

75 x 10 = EUR 750.00 b2
remaining sheets additional amount

Add amounts entered at b1 and b2 and enter total at B EUR 1,163.00 B

Designation Fees

The international application contains 83 designations.

10 x 95 = EUR 950.00 D
number of designation fees amount of designation fee
payable (maximum 10)

Add amounts entered at B and D and enter total at I EUR 2,113.00 I
(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT (if applicable) EUR 30.00 P

5. TOTAL FEES PAYABLE EUR 3,190.00
Add amounts entered at T, S, I and P, and enter total in the TOTAL box
TOTAL

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

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☐ cheque

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☒ (this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☒ is hereby authorized to charge the fee for preparation and transmission of the priority document to the International Bureau of WIPO to my deposit account.

2800.0321

12 November 1999

DR. Hans-Rainer Jaenichen

Deposit Account No.

Date (day/month/year) (Jae/PST/hä)

Signature

European Patent Attorney

Claims

1. A recombinant DNA molecule comprising:
- (i) a nucleic acid molecule encoding a subtilisin-like serine protease or encoding a biologically active fragment of such a protein, selected from the group consisting of
 - (a) nucleic acid molecules comprising a nucleotide sequence encoding a protein comprising the amino acid sequence as given in SEQ ID NO: 2, 8, 10 or 12;
 - (b) nucleic acid molecules comprising a nucleotide sequence as given in SEQ ID NO: 1, 7, 9 or 11;
 - (c) nucleic acid molecules encoding a protein comprising at least the D region, H region, substrate binding site and/or S region of the subtilisin-like serine protease encoded by a nucleic acid molecule of (a) or (b); or
 - (d) nucleic acid molecules hybridizing with the complementary strand of a nucleic acid molecule as defined in any one of (a) to (c);
 - (e) nucleic acid molecules encoding a protein the amino acid sequence of which is at least 65% identical to the amino acid sequence encoded by a nucleic acid molecule of any one of (a) to (c);
 - (f) nucleic acid molecules; the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of a nucleic acid molecule as defined in any one of (a) to (e); or
 - (ii) a nucleic acid molecule encoding a mutant non-active or a hyper-active form of or an antibody against the subtilisin-like serine protease encoded by a nucleic acid molecule of (i); or
 - (iii) a nucleic acid molecule which specifically hybridizes with a nucleic acid molecule of (i) or the complementary strand thereof.

REPLACED BY
ART 34 AMDT

2. The recombinant DNA molecule of claim 1 wherein the nucleic acid molecule is DNA, cDNA, genomic DNA or synthetically synthesized DNA.
3. The recombinant DNA molecule of claim 1 wherein the nucleic acid molecule is derived from a plant, preferably Arabidopsis or potato.
4. The recombinant DNA molecule of any one of claims 1 to 3 wherein said nucleic acid molecule is operably linked to regulatory elements allowing the expression of the nucleic acid molecule in plants.
5. A vector comprising a recombinant DNA molecule of any one of claims 1 to 4.
6. A host cell containing a vector of claim 5 or a recombinant DNA molecule of any one of claims 1 to 4.
7. A method for the production of transgenic plants with altered stomata characteristics compared to wild type plants comprising the introduction of a recombinant DNA molecule of any one of claims 1 to 4 or the vector of claim 5 into the genome of a plant, plant cell or plant tissue.
8. A transgenic plant cell comprising stably integrated into the genome a recombinant DNA molecule of any one of claims 1 to 4 or a vector of claim 5 or obtainable according to the method of claim 7, wherein the expression of the nucleic acid molecule results in an increased expression or activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.
9. A transgenic plant or a plant tissue comprising plant cells of claim 8.
10. The transgenic plant of claim 9 which displays a decreased stomata density, lower conductance of stomata and/or wherein the water consumption is lowered compared to wild type plants.

11. A transgenic plant cell which contains stably integrated into the genome a recombinant DNA molecule of any one of claims 1 to 4 or part thereof, a vector of claim 5 or obtainable according to the method of claim 7, wherein the presence, transcription and/or expression of the nucleic acid molecule or part thereof leads to reduction of the synthesis or the activity of subtilisin-like serine proteases in transgenic plants compared to wild type plants.
12. The plant cell of claim 11, wherein the reduction is achieved by an antisense, sense, ribozyme, co-suppression and/or dominant mutant effect.
13. A transgenic plant or plant tissue comprising the plant cells of claim 11 or 12.
14. The transgenic plant of claim 13 which displays increased stomatal density and/or higher conductance of stomata and/or increased content of sugars and/or protein in plant leaves compared to wild type plants.
15. Harvestable parts or propagation material of plants of any one of claims 9, 10, 13 or 14 comprising plant cells of claim 8, 11 or 12.
16. A kit comprising a recombinant DNA molecule of any one of claims 1 to 4 or a vector of claim 5.
17. A method for the production of a transgenic plant comprising an increased yield and/or increased stomatal density compared to wild type plants, wherein
 - (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in a decreased activity of a subtilase;
 - (b) a plant is regenerated from the cell prepared according to step (a); and
 - (c) further plants, if any, are generated from the plant prepared according to step (b).

18. A method for the production of a transgenic plant having a decreased water consumption and/or decreased stomatal density compared to wild type plants wherein
 - (a) a plant cell is genetically modified by the introduction of a foreign nucleic acid molecule the presence of which or the expression of which results in an increased activity of a subtilase;
 - (b) a plant is regenerated from the cell prepared according to step (a); and
 - (c) further plants, if any, are generated from the plant prepared according to step (b).
19. Use of a nucleic acid molecule encoding or regulating the expression of a subtilisin-like serine protease or a nucleic acid molecule hybridizing with such a nucleic acid molecule, a nucleic acid molecule as defined in any one of claims 1 to 4, a recombinant DNA molecule of any one of claims 1 to 4, or a vector of claim 5 for the production of plants with improved fresh and dry weight, for enhancing the content of sugars and/or protein in plant leaves for the production of plants with reduced leaf temperatures or with reduced water loss and lower water consumption, for the modulation (enhancement) of CO₂ uptake into and H₂O release from leaves, for sustained photosynthesis under high intensity conditions or for the improvement of disease resistance of plants.